REMARKS

A. Status of the Claims

Claims 1-4 are currently pending and under consideration. Claims 5-15 were previously withdrawn. Claims 16-23 have been added. Support for these claims can be found, at least, in the Specification on page 4, lines 10-21; page 6, lines 4-17; and Examples 1-5. No new matter is added. The Office is authorized to deduct any additional claim fees due from the deposit account of Applicants' representative, which is identified on page 1 of this paper.

B. Rejections Under 35 U.S.C. § 103(a)

(1) Claims 1-4 are rejected under 35 U.S.C. § 103(a) as obvious over Enriquez-Obregon *et al.* (1997, *Biotecnologia Aplicada,* 14:169-174) in view of Packer *et al.* (*Free Rad. Biol. Med.*, 19:227-250, 1995). In particular, the Action asserts that Enriquez-Obregon *et al.* disclose a method for transforming a plant cell by culturing the cell on medium comprising an antioxidant, wherein the presence of the antioxidant increased explant viability and that Packer *et al.* disclose lipoic acid as a biological antioxidant. It is thus asserted that it would be obvious to modify the method of using an antioxidant for increasing transformation efficiency of Enriquez-Obregon *et al.* to use lipoic acid in view of Packer *et al.* Applicants respectfully traverse for at least the reasons described below.

a. The Cited References Fail to Suggest the Claimed Invention

The claimed invention relates to a method for introducing a nucleic acid sequence into the genome of a plant cell and regenerating a transformed plant therefrom, comprising the use of a plant transformation media with lipoic acid in an amount

effective for increasing the efficiency of transformation or regeneration. Enriquez-Obregon *et al.* discloses sugarcane transformation with media containing a very specific combination of antioxidants comprising ascorbic acid, cysteine, and silver nitrate. Packer *et al.* generally relates to culturing mammalian cells.

One of skill in the art would have no expectation of any benefit or success in combining the teachings of Enriquez-Obregon *et al.* and Packer *et al.* with respect to transforming a plant cell and/or regenerating a plant therefrom on at least one plant transformation media comprising lipoic acid. Enriquez-Obregon *et al.* provides no teaching regarding the general use of antioxidants for transformation and, in fact, only discloses the use of a ***very specific antioxidant combination*** with no indication that any substitute for the disclosed combination would be beneficial or even successful. Nor does either reference teach that lipoic acid would be suitable for plant culture and genetic transformation or an appropriate substitute for the antioxidant combination of Enriquez-Obregon *et al.* There would therefore be no expectation that the specific antioxidant combination of Enriquez-Obregon *et al.* (ascorbic acid, cysteine and silver nitrate) could successfully be replaced with lipoic acid.

Further, the reference provides no reason for one of skill in the art to believe that any other antioxidant, either singly or in combination, would be necessary, beneficial or even successful. Nor has the Examiner provided any indication that one would desire a different antioxidant than the combination disclosed. Without such desire one of skill in the art would have no reason to deviate from the antioxidant preparation proven to work effectively in Enriquez-Obregon *et al.* and thus would have no reason to substitute lipoic acid for the specific combination disclosed in Peri *et al.*

Moreover, Packer *et al.* discloses the use of lipoic acid in relation to culturing ***mammalian cells*** and does not teach any connection between lipoic acid for use with plant cells or plant genetic transformation. It is well known that transformation methods vary greatly between mammalian and plant cells and thus methods appropriate for culturing mammalian cells would be expected to differ substantially from that useful for plant transformation. One of skill in the art would therefore not expect that lipoic acid, disclosed for use in mammalian cell culture in Packer *et al.*, would be a suitable substitution as an antioxidant in plant cell culture, let alone as a substitute for the combination of antioxidants disclosed in Enriquez-Obregon *et al.*

b. The Claimed Invention Yields Unexpected Results

The surprising and unexpected results shown in the Applicant's Specification further demonstrate the non-obviousness of the claimed invention. Specifically, the working examples of the specification show that the claimed invention results in an increase in transgenic plant production, heterologous gene expression, transformation efficiency and a decrease in tissue browning. This would in no way be predicted by one of ordinary skill in the art as of the filing date and thus these unexpected results establish the non-obviousness of the claimed invention.

For instance, the working examples of the current application demonstrate multiple transformation and regeneration benefits in five distinct types of plants (tomato, potato, wheat, soybean and cotton). In particular, Example 1 shows the effects of lipoic acid on a cultivar of tomato species, *Lycopersicon esculentum* on browning, transient expression of a heterologous reporter gene, number of transgenic plants and events produced per explant, reduction of non-transgenic shoots (escapes), number of shoots

produced per responding explant, the percentage of responding explants producing transgenic plants, and the percentage of transgenic plants produced per shoot.

In particular, as shown in Tables 1 and 3, on pages 31 and 33 of the Specification, tissue browning in transgenic tomato was measured in and around a poked region on the cotyledon explant. All treatments of lipoic acid at concentrations of 5, 10, 50, and 100uM demonstrated about a ***2 fold reduction*** of tissue browning and had a ***1.5 to 1.8 fold increase*** in the number of explants with less than 30% tissue browning as compared to the treatment without lipoic acid.

The impact of lipoic acid on expression of the marker gene beta-glucuronidase (GUS) is shown in Tables 2 and 3, on pages 32 and 33 of the Specification. Treatments of lipoic acid at concentrations of 10 and 50uM ***doubled*** the percentage of explants having high transient expression (at least 30% of poked regions producing GUS blue spots) to up to 60.9%. Additionally, when lipoic acid was used at concentrations of 10, 50 and 100uM, ***100%*** of explants transiently expressed the GUS gene**.**

Similarly, the use of lipoic acid yielded an increase in the percentage of transgenic plants produced per explant by ***2.7 to 4.3 fold***, as well as an increase in transgenic events produced per explant by ***1.8 to 3.4 fold***. The impact of lipoic acid on the percentage of transgenic plants and transgenic events produced per explant is shown in Table 3, on page 33 of the Specification. In particular, treatment with lipoic acid increased the percentage of transgenic plants produced per explant from ***40.1% to 178.7%*** and increased the percentage of transgenic events produced per explant from ***27.9% to 94.1%.***

The number of non-transgenic shoots (escapes) was also ***reduced by 1.5 to 1.6 fold*** when lipoic acid was used. In particular, culturing with lipoic acid decreased

escapes from ***88.1% to as low as 54.6%***.

The effect of lipoic acid on shoot induction and growth is also shown. For instance, Table 4, on page 34 of the Specification, shows that lipoic acid produced a ***1.2 to 1.7 fold increase*** in the number of shoots produced per responding explant. Similarly, as shown in Tables 5 and 6, on pages 35 and 36 of the Specification, lipoic acid increased the percentage of responding explants that generated transgenic plants from ***2 to 4 fold*** and increased the number of transgenic plants per responding explant from ***4.0 to 7.4 fold***. Table 5 further shows a ***3.6 to 4.3 fold increase*** in the percentage of transgenic plants produced per shoot compared to treatment without lipoic acid.

Similar effects were seen in lipoic acid treatments of a potato cultivar, Ranger Russet. Results in Table 7, on page 39 of the Specification, depict a ***6 fold increase*** in transformation efficiency, from ***3% to as great as 19%***. Lipoic acid also reduced the percentage of escapes (non-transgenic shoots) from ***50% in the control to 16%***.

Positive results are also shown in the working examples for wheat (Example 3), soybean (Example 4) and cotton (Example 5). For instance, Table 9 of Example 3, on page 43 of the Specification shows that lipoic acid at a concentration of 50μM in the delay, selection, and regeneration media resulted in an increase in the percentage of responding wheat calli from ***32.5% to 47.9%*** and increased efficiency from ***3.0% to 5.1%***. When different concentrations in the delay, selection and first regeneration media (Table 10, on page 43 of the Specification) were evaluated, 25μM in the delay medium, 50μM in the selection medium, and 50μM in the first regeneration medium demonstrated an increase in transformation efficiency from ***2.9% to 5.4%***.

Similarly, soybean transformation using lipoic acid at concentrations of 250μM to

1500μM demonstrated increased transformation efficiency and shoot and rooted plant production. In particular, as shown in Table 12, on page 46 of the Specification, treatment with lipoic acid increased transformation efficiency from ***0.6% to as great as 3.7%.***

Finally, Table 13, on page 50 of the Specification, shows that treatment of cotton cultures with lipoic acid in the selection stage increased the frequency of embryogenic callus formation from ***41.4% to as great as 61.2%.***

The foregoing results represent significant improvements in transformation and regeneration in multiple plant types that would in no way have been expected based on the knowledge in the art. Nothing in the art suggests lipoic acid as a suitable antioxidant for use in plant transformation and regeneration, let alone the significant benefits shown in the Specification, thus the above results are unexpected.

These unexpected results, along with the failure of the cited art to provide a reasonable expectation of success, demonstrate the non-obviousness of the claimed invention. Withdrawal of the rejection is therefore respectfully requested.

(2) Claims 1-4 are rejected under 35 U.S.C. § 103(a) as obvious over Peri *et al.* (*Nature Biotechnol.*, 14:624-628, 1996) in view of Packer *et al.* (*Free Rad. Biol. Med.*, 19:227-250, 1995). In particular, it is asserted that Peri *et al.* disclose a method of transforming grape cells and regenerating a transformed plant therefrom by culturing the plant cell on a media containing the antioxidant polyvinylpolypyrrolidone (PVPP), wherein the antioxidant increased the number of viable calli, plants obtained and transformation efficiency. Packer *et al.* is asserted to disclose lipoic acid as a biological antioxidant. Applicants respectfully traverse for at least the reasons described below.

a. The Cited References Fail to Suggest the Claimed Invention

The claimed invention relates to a method for introducing a nucleic acid sequence into the genome of a plant cell and regenerating a transformed plant therefrom, comprising the use of a plant transformation media with lipoic acid in an amount effective for increasing the efficiency of transformation or regeneration. Peri *et al.* is cited as disclosing the use of multiple antioxidants, alone and in combination, during transformation of grape plants to increase explant viability and Packer *et al.* generally is cited for teaching relating to culturing mammalian cells.

One of skill in the art would have no expectation of any benefit or success in combining the teachings of Peri *et al.* and Packer *et al.* with respect to the claimed invention. In particular, although Peri *et al.* mentions an antioxidant combination of polyvinylpolypyrrolidone (PVPP) and dithiothreitol (DTT) that improves plant viability, ***ten other antioxidant preparations showed minimal to no effect*** (*see* Peri *et al.* page 625, first full paragraph). Peri *et al.* thus provides no teaching that antioxidants in general would successfully increase plant viability or transformation efficiency. One of skill in the art would therefore have no expectation that the specific combination of PVPP and DTT could successfully be replaced with lipoic acid. Furthermore, Peri *et al.* provides no teaching or suggestion that any antioxidant other than the disclosed PVPP and DTT combination would be necessary, beneficial or even successful. Nor does the Action show that one of skill in the art would have any desire to modify the methods disclosed in Peri *et al.* to find an antioxidant suitable to replace those shown to be sufficient.

In addition, neither reference teaches that lipoic acid would be suitable for plant

culture and genetic transformation or that it is an appropriate substitute for the antioxidant combination disclosed in Peri *et al.* In particular, Packer *et al.* discloses the use of lipoic acid in relation to culturing ***mammalian cells*** and does not teach any connection between lipoic acid for use with plant cells or plant genetic transformation. It is well known that transformation methods vary greatly between mammalian and plant cells and thus methods useful for mammalian transformation may not be useful for plant transformation. One of skill in the art would therefore not expect that lipoic acid, disclosed for use in mammalian cell culture in Packer *et al.*, would be a suitable substitution as an antioxidant in plant cell culture, let alone as a substitute for the combination of antioxidants disclosed in Peri *et al.*

b. Peri *et al.* Teach Away From the Claimed Invention

Although the Action asserts that it would have been obvious to one of ordinary skill in the art to modify the method of using an antioxidant for increasing transformation efficiency taught by Peri *et al.*, the reference teaches that the use of antioxidants in transformation methods is highly unpredictable. For instance, Peri *et al* discloses the use of a specific combination of two antioxidants (PVPP and DTT) in transformation methods to increase explant viability. Ten other antioxidant media preparations were also tested, however, ***only the single PVPP and DTT combination was sufficiently effective***, while 2 individual antioxidant preparations demonstrated lower than acceptable viability results and ***eight antioxidant preparations produced no increase in*** **viability**. Peri *et al.* therefore clearly teaches away from substituting one antioxidant for another due to the observed variability and unpredictability between the numerous antioxidants tested.

In addition to the unpredictability of antioxidants, Peri *et al.* also teaches that the antioxidants, although increasing viability, ***do not increase plant transformation efficiency*** and thus teaches away from the claimed invention. In particular, the reference discloses the transformation of tobacco plant explants with *Agrobacterium* comprising a GUS-Intron plasmid co-cultured on media containing the antioxidants PVPP and/or DTT. The authors state that such co-culturing demonstrates that "***[t]he same percentage*** of tobacco GUS expressing explants were obtained with and without the addition of these antioxidants." (Peri *et al.*, page 625, second column, first full paragraph, emphasis added). Peri *et al.* therefore discloses that the same antioxidants taught to increase explant viability during transformation of grapes fail to increase transformation efficiency in other plants, such as tobacco. This disclosure clearly teaches away from claimed use of lipoic acid to increase transformation and/or regeneration efficiency.

c. The Claimed Invention Yields Unexpected Results

As described above, the Applicant's Specification shows surprising and unexpected results that would not be predictable in view of the cited art and thus further establish non-obviousness. In particular, the Working Examples demonstrate increased transformation and regeneration efficiency of 5 different plants (tomato, potato, wheat, soybean and cotton). For instance, as described in detail above, Tables 1-5 in Example 1 show a 2 fold decrease in tissue browning, a 2 fold increase in transient expression, a 6.5 fold increase in transformation efficiency and an increase in shoot growth and development in tomato.

Table 7, in Example 2 shows a 6 fold increase in transformation efficiency and decrease in escapes from 50% to 16% in potato. Tables 9 and 10 in Example 3 show an

increase in the percentage of responding calli from 32.5% to 47.9%, and an increase in transformation efficiency from 3.0% to 5.1% or 2.9% to 5.4% with optimized concentrations in delay, selection and regeneration media in wheat. Table 12 in Example 4 shows an increase in transformation efficiency from 0.6% to as great as 3.7% in soybean and Table 13, in Example 5 shows an increase in the frequency of cotton embryogenic callus formation from 41.4% to 61.2%.

The results described above would in no way be predictable in view of the cited art. This is especially true in view of the disclosure in Peri *et al.* teaching away from the use of lipoic acid in transformation efficiency in tobacco plants and the failure of Packer *et al.* to disclose any teaching or suggestion for the use of lipoic acid for culturing of plant cells in general.

These unexpected results, failure of the cited references to provide a reasonable expectation of success and the teaching away of Peri *et al.* clearly demonstrates the non-obviousness of the claimed invention. Withdrawal of the rejection is thus respectfully requested.

(3) Claims 1-4 are rejected under 35 U.S.C. § 103(a) as obvious over Cai *et al.* (U.S. Patent No. 6,369,298) in view of Packer *et al.* (*Free Rad. Biol. Med.*, 19:227-250, 1995). In particular, it is asserted that Cai *et al.* disclose a method of transforming sorghum cells and regenerating a transformed plant therefrom by culturing the plant cell on a media containing an antioxidant, wherein the antioxidant increased the transformation efficiency. Packer *et al.* is asserted to disclose lipoic acid as a biological antioxidant. Applicants respectfully traverse for at least the reasons described below.

a. The Cited References Fail to Suggest the Claimed Invention

Cai *et al.* is cited as teaching transformation of sorghum primarily without the addition of antioxidants in the media and (briefly) describing the use of a single antioxidant (PVPP) added to the co-cultivation medium. Packer *et al.* is again cited for teachings related to culturing mammalian cells.

The Cai *et al.* reference refers to the Peri *et al.* reference, discussed above, as motivation to add PVPP to the disclosed co-culture medium. However, as detailed in the response to the rejection over Peri *et al.* above, the reference teaches that the use of antioxidants to increase transformation efficiency is ***highly unpredictable***. Neither Peri *et al.* nor Cai *et al.* teach or suggest that antioxidants in general increase transformation or regeneration efficiency or that any antioxidant other than those disclosed would be necessary. One of skill in the art would therefore have no expectation of any benefit or success in combining the teachings of Cai *et al.* and Packer *et al.* by modifying the method of Cai *et al.* to substitute lipoic acid.

Additionally, neither Cai *et al.* nor Packer *et al.* teach or suggest that lipoic acid would be suitable for plant culture and genetic transformation or that it is an appropriate substitute for the antioxidant disclosed in Cai *et al.* In particular, as described in the rejections above, Packer *et al.* discloses the use of lipoic acid in relation to culturing ***mammalian cells*** and does not teach any connection between lipoic acid for use with plant cells or plant genetic transformation. Due to the known vast differences between mammalian and plant cell culture and transformation techniques, one of skill in the art would have no expectation that methods or media successful in one would be successful in the other. There would therefore be no expectation that lipoic acid, disclosed for use in

mammalian cell culture in Packer *et al.*, would be a suitable substitution as an antioxidant in plant cell culture.

b. The Claimed Invention Yields Unexpected Results

The non-obviousness of the claimed invention is further demonstrated by the unexpected results yielded by the claimed invention. For instance, Applicant's Specification demonstrates an increase in shoot production and transformation efficiency in multiple plant types. For example, a 2 fold decrease in tissue browning, a 2 fold increase in transient expression, a 6.5 fold increase in transformation efficiency and an increase in shoot growth and development were observed in tomato and similar positive results were seen in potato, wheat, soybean and cotton (*see* working Examples 1-5 and Tables 1-5, 7 and 9, 10, 12 and 13). These results would in no way be predictable in view of the cited art.

The foregoing therefore clearly demonstrates the non-obviousness of the claimed invention. Withdrawal of the rejection is thus respectfully requested.

(**4**) Claims 1-4 are rejected under 35 U.S.C. § 103(a) as obvious over Ciccarone *et al.* (U.S. Patent Application Publication No. 2003/0096414). In particular, it is asserted that Ciccarone *et al.* disclose a method of transforming a plant cell and culturing the cell on medium comprising valeric acid and that lipoic acid is an example of valeric acid. Applicants respectfully traverse for at least the reasons described below.

a. The Cited References Fail to Teach or Suggest All Elements of the Claimed Invention

The cited references, either in combination or alone, fail to teach or suggest the claimed subject matter. For instance, claim 1 recites:

> A method for introducing a nucleic acid sequence into the genome of a plant cell and regenerating a transformed plant therefrom, said method comprising:
>
> a) ***transforming a plant cell***; and
>
> b) regenerating a transformed plant therefrom, wherein the transforming and/or regenerating comprises culturing said plant cell on at least one plant transformation media, said at least one plant transformation media comprising an amount of lipoic acid or an analog thereof effective for ***increasing the efficiency of the transformation and/or regeneration of a plant therefrom***.

However, neither Ciccarone *et al.* nor Packer *et al.* disclose transforming a plant cell and regenerating a plant therefrom, wherein transforming or regenerating comprises culturing the plant cell on at least one plant transformation media comprising lipoic acid or an increase in transformation or regeneration efficiency of a plant, as required by claim 1.

In particular, Ciccarone *et al.* is generally directed to the culturing and transfection of ***mammalian epithelial cells***. The only mention of the use of plant cells is in paragraph 120 stating the disclosed culture medium may be used to culture plant and/or animal cells. The reference, however, continues to state that it is preferably used for culturing mammalian cells. In fact, the reference provides no examples, working or prophetic, regarding plant cell cultures and no disclosure at all of plant transformation. Ciccarone *et al.* therefore clearly does not disclose the use of lipoic acid for plant transformation or to increase transformation or regeneration efficiency. As described above, Packer *et al.* does not remedy this deficiency, as the references does not disclose

transformation or regeneration of plant cells, but instead also generally relate to culturing ***mammalian cells***. The combination of the claimed references therefore does not teach or suggest the use of lipoic acid in **a *plant* *transformation method to increase transformation or regeneration efficiency***.

Additionally, the only mention of lipoic acid in Ciccarone *et al.* is in relation to its use in media for ***mammalian cell culture.*** For instance, regarding the extensive laundry list of contemplated media components, including lipoic acid, in paragraphs 110-113, the reference states that "each ingredient is present in an amount which supports the suspension cultivation of a mammalian epithelial cell *in vitro*." (Ciccarone *et al.,* paragraph 113). Tables 1-3 in the cited reference also disclose possible culture media ingredients, including lipoic acid. However, paragraph 114 describes the media disclosed in Tables 1-3 as "suitable for use in the culture of a variety of mammalian cells." (Ciccarone *et al.,* paragraph 114). In fact, Example 4 (Table 7), directed to transfection of human HEK epithelial cells (293-F cells), is the only example mentioning lipoic acid. One of skill in the art would therefore have no expectation that the culturing and transfection techniques for mammalian cells as disclosed in Ciccarone *et al.* and Packer *et al.* could be successfully modified for plant transformation.

b. The Claimed Invention Yields Unexpected Results

Furthermore, the invention yields the surprising and unexpected results described above. Such unexpected results include the disclosed increase in shoot production and transformation efficiency, an increase in transient expression of a heterologously introduced marker gene, and a decrease in tissue browning when lipoic acid was used in transformation media (*see* working Examples 1-5 and Tables 1-3, 7 and 9-13).

These results, in combination with the failure of the references, either in combination or alone, to teach or suggest the claimed subject matter, clearly demonstrate the non-obviousness of the claimed invention. Withdrawal of the rejection is therefore respectfully requested.

C. Conclusion

In light of the foregoing, Applicants submit that all claims are in condition for allowance, and an early indication to that effect is earnestly solicited. The Examiner is invited to contact the undersigned at (214) 259-0931 with any questions, comments or suggestions relating to the referenced patent application.

Respectfully submitted,

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